Journal of Surgical Oncology 2000;73:164–171

Tumoricidal Effects of Onconase on Various Tumors

INTAE LEE, PhD,[1]* YOUNG H. LEE, BA, STANISLAW M. MIKULSKI, MD,[2] JIHEAN LEE, KENNETH COVONE, BS, AND KUSLIMA SHOGEN, PhD[2]

[1]*Department of Radiation Oncology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania*

[2]*Alfacell Corporation, Bloomfield, New Jersey*

Background and Objectives: The effects of Onconase (Onc) on the tumor growth in vitro and in vivo were examined. Because elevated tumor interstitial fluid pressure (TIFP) is one of the major causes of inadequate drug delivery into solid tumors, we tested if Onc could lower TIFP in solid tumors.

Methods: We used several assays including a clonogenic assay and a growth delay assay for the determination of anti-tumoricidal effects of Onc. We also measured Onc-induced changes in several tumor physiological parameters.

Results: Onc demonstrated cytotoxic effects in all eight exponentially growing cell lines in vitro. It effectively inhibited the growth of all four transplanted tumors in vivo, and significantly reduced TIFP in all four tumors. Onc also induced increases in tumor blood flow (TBF) as well as increases in median tumor oxygen partial pressure (pO_2) in solid tumors.

Conclusions: Onc showed anti-tumoral effects on various tumor cells in vitro as well as in vivo. We also gained some insight regarding the potential physiological benefit of Onc as a new therapeutic agent in cancer treatment. Due to increases in both TBF and tumor pO_2, Onc could be a potential candidate as a novel radiation enhancer; therefore, the study of the radiation response in vivo is warranted.

J. Surg. Oncol. 2000;73:164–171.

KEY WORDS: **Onconase; cytotoxicity; tumor pathophysiology; solid tumors.**

INTRODUCTION

Although the mechanisms of the cell destruction by RNase are poorly understood, Wu et al. [1] reported that the P-30 protein, a member of the RNase A superfamily, showed cytotoxic effects against 9L glioma cells in vitro. Onconase (Onc), previously known as the P-30 protein, is a 12-kDa protein homologous to pancreatic RNase A and is isolated from amphibian oocytes. Onc binds to the membranes on the cell surface, enters the cell cytosol, and degrades RNA, causing cell death. Unlike RNase A, the mechanism of protein synthesis inhibition by Onc apparently does not involve degradation of lysate or cellular ribosomal RNAs, but acts by inactivating cellular tRNA [2]. Since the initiation of clinical trials, very limited in vivo data are available. Mikulski et al. [3] reported that the efficacy of the P-30 protein delayed the time of death in BALB/C mice bearing intraperitoneal implants of M109 mouse lung carcinoma. The anticancer effect of P-30 protein appeared to be associated with its RNase activities. However, no other solid tumor models were tested for the anti-tumor effectiveness of Onc in vivo.

Although the etiology of the elevated tumor interstitial fluid pressure (TIFP) in solid tumors (one of the physi-

Young H. Lee, Jihean Lee, and Kenneth Covone worked at the Radiation Research Laboratory, Division of Radiation Research, UMDNJ-Robert Wood Johnson Medical School, Camden, NJ 08103. In December 1998, this laboratory was closed, due to the lack of research funds.

*Correspondence to: Intae Lee, PhD, Department of Radiation Oncology, 195 John Morgan Building, 3620 Hamilton Walk, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6072. Fax: (215) 898-0090. E-mail: ilee2@mail.med.upenn.edu

Accepted 5 October 1999

ological barriers for drugs and macromolecules) is not fully understood [4,5], several TIFP-lowering strategies have been devised. The reduction in TIFP by physical and chemical modifiers is important for cancer diagnosis and has value in prognostication [6–9]. A decrease in TIFP in responding tumors is documented in patients with carcinoma of the uterine cervix during radiation therapy [9], and in patients with melanoma and lymphoma following interleukin-2 (IL-2) therapy [6]. Therefore, we examined whether Onc could effectively lower the TIFP in solid tumors. Thus, we measured TIFP using the wick-in-needle (WIN) method, tumor blood flow (TBF) using laser Doppler flowmetry, and intratumor oxygen partial pressure (pO_2) using a needle-type microelectrode method before, during, and after treatments with Onc. These experiments have provided some insight regarding the potential physiological benefits of Onc as a new therapeutic agent in cancer treatments.

MATERIALS AND METHODS

Tumor and Endothelial Cells

The six cell lines (CCL-209 bovine endothelial cells, DU145 human prostate carcinoma, Ls174T human colon adenocarcinoma, $HepG_2$ human hepatoma, AsPC-1 human pancreas adenocarcinoma, H4IIE rat hepatoma) were purchased from the American Type Culture Collection (Rockville, MD). The original FSaII (murine fibrosarcoma) and MCaIV (murine adenocarcinoma) tumor cell lines were obtained from Dr. L. Gerweck (Massachusetts General Hospital, Boston, MA). The cells were quickly thawed at 37°C in a water bath and maintained at this temperature for the duration of the experiments. The cells grew either exponentially or confluently (plateau-phase: confluent level is >90%) in vitro in various culture media (i.e., Dulbecco's modified Eagle medium [DMEM], modified Eagle medium [MEM], and Rosewell Park Memorial Institute Medium [RPMI 1640]) in either 10–20% of fetal bovine serum (FBS; certified, heat-inactivated: GIBCO BRL, Grand Island, NY) or 10% of calf serum (GIBCO BRL).

Preparations of Onc

Onc was supplied by the Alfacell Corporation (Bloomfield, NJ). Original stock solutions of Onc 1 mg/ml were made in sterile distilled water, and frozen into a −20°C freezer until needed. Onc was thawed and diluted to the proper concentration prior to the experiments. The alkylation of Onc was done with iodoacetate, which dramatically decreased its ribonucleic activity. Thus, the ribonucleolytic activity retained only 2% effectiveness in vitro after the alkylation [1].

Clonogenic Assay

The proper cell number (2×10^5) was preincubated in T-25 cell culture flasks overnight, then incubated with Onc at 0–20 μg/ml for 24–72 h prior to the performance of the clonogenic assay. After exposure to Onc, clonogenic assay was performed as described [10].

MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-tetrazolium bromide) Assay

The proper cell number (5×10^4 to 1×10^5) was preincubated in 24-well cell culture plates overnight, then treated with fresh media containing 0–20 μg/ml of Onc. After 24 h exposure to Onc, Onc was removed. After 2 days, the MTT (Sigma, St. Louis, MO) assay was performed. The MTT colorimetric assay was performed using a Dynatech MR-5000 plate reader (Dynatech Laboratories, Chantilly, VA). This test was based on the selective ability of live cells to reduce the yellow soluble salt of MTT to the purple-blue insoluble formazan precipitate [11]. Experiments were performed in 96-well cell culture plates. MTT was dissolved in phosphate buffered saline at 5 mg/ml.

Cell Viability by Trypan Blue Exclusion

The proper cell number (5×10^4 to 1×10^5) was preincubated in 24-well cell culture plates overnight, then treated with fresh media containing 0–25 μg/ml of Onc. After 24 h exposure to the drug, adherent cells were trypsinized and added to the corresponding centrifuge tubes. The suspensions of cells were then diluted 1:1 with 0.25% trypan blue (Sigma) and immediately scored for viability in a hemacytometer.

Animals Bearing Tumors

The institution's guidelines for the care and use of laboratory animals were followed. Female C3H mice 8–10 weeks old (purchased from the Cox animal facility, Massachusetts General Hospital), bearing either FSaII murine fibrosarcoma or MCaIV murine mammary adenocarcinoma, were utilized. Ten-week-old female rats were used for transplantation of H4IIE rat hepatoma. Nude mice were used for the implantation of DU145 human prostate carcinoma. Animals were kept under pathogen-free conditions in the vivarium maintained at 25 ± 3°C. They were allowed food and water ad libitum.

Single cell suspensions were prepared using 0.25% trypsin solution. Either ~2×10^5 (i.e., FSaII or MCaIV) or ~1×10^6 (i.e., H4IIE, DU145) viable cells suspended in 50 μl of DMEM were injected subcutaneously (sc) into the right thighs of either mice or rats. Most experiments were carried out when the leg tumor volume was ~250 mm^3 for mice and ~750 mm^3 for rats. Tumor volumes were calculated using the formula $V = 0.4 \times ab^2$,

where a and b were the longer and shorter perpendicular diameters of the tumor, respectively [5].

Anesthesia

For the measurements of tumor physiological parameters, either mice or rats were anesthetized with a mixture of ketamine (90 mg/kg) and xylazine (9 mg/kg) via the intramuscular (im) route. Animals were placed on a heating pad to keep the body temperature at ~37.5°C. Their rectal temperature was monitored by using a Type-T thermocouple and BAT-10 thermometer (Physitemp, Clifton, NJ) [12].

Onc Treatment In Vivo

Onc was dissolved in sterile saline before the experiments. When the tumor reached the proper size, the mice were given single or multiple intraperitoneal (ip) injections of Onc 10 mg/kg (200 μg per 20 g mouse) with a volume of 0.2 ml. Experiments to measure the pathophysiological parameters for the acute effects of Onc were carried out when the tumor volume reached ~250 mm^3. To determine the effects of chronic treatment, C3H mice were treated with Onc every 3 days (days 0, 3, 6) when the tumor reached ~200 mm^3 in volume. Nude mice bearing DU145 tumors were treated with a single ip injection of Onc 5 mg/kg (100 μg per 20 g mouse).

Measurements of Mean Arterial Blood Pressure (MABP) and TIFP

MABP was measured by a carotid artery cannulation technique [12]. TIFP was measured with the WIN technique using 23-gauge needles with a side hole 2 mm from the tip. Then, all TIFPs were measured at 1 h prior to day 0 and days 1, 4, and 7 after the treatment. In separate experiments, TIFP and MABP were measured during 1 h of observation after treatment with Onc. Measurements were made by introducing WIN needles into the central regions of the tumors using a MacLab/ 4e analog digital system (ADInstruments, Milford, MA) linked to a Macintosh computer [12].

Measurements of Blood Perfusion Using Laser Doppler Flowmetry

Red blood cell (RBC) flux was measured using the laserflow blood perfusion monitor-BPM 403A (Vasamedic, St. Paul, MN) with a 0.8-mm diameter laser Doppler needle probe inserted in the tumor center using a 23-gauge needle. The electrical signals of flow, velocity, and volume from the laser Doppler systems were digitally processed using a MacLab system linked to a Macintosh computer with output voltage ranging from 0 to 2.5 V [12].

Measurements of Tumor pO_2

Polarographic measurements of tumor pO_2 were performed using a 27-gauge needle type electrode of pO_2 (membranized recessed cathode, sensor electrode diameter = 25 μm; General Diamond, Ann Arbor, MI). The temperature of the pO_2 calibration solution was maintained at ~34°C using a calibration cell mounted on a water bath. The electrodes were calibrated by immersion in a series of isotonic saline solutions saturated with five different known O_2 concentrations. About 40 measurements were made for each tumor [12].

Statistical Evaluation

All measured values are shown as the mean ± SE or the median of each group for the statistical evaluation. Percent changes were determined individually for each mouse, based on pretreatment values, and then averaged. Significant differences within a group before and after Onc treatment were evaluated using a paired *t*-test, and between treatment groups with an unpaired *t*-test. Tumor pO_2 histograms were tested for significant differences in the two independent groups using a Mann-Whitney U-test. $P < 0.05$ was considered significant.

RESULTS

To determine the inhibitory effect of Onc on cell growth, cell viability (trypan blue exclusion assay) as well as MTT assay were performed after 24 h exposure to Onc. When the cell viability was determined immediately after exposure to Onc using the trypan blue exclusion assay, we observed that there were no changes in cell numbers or viability. Similar results were observed from the MTT assay of eight cell lines under the same conditions. Insignificant changes were observed when the MTT assay was performed after 24 h exposure to Onc. However, after exposure to Onc for 24 h, followed by Onc removal and varying times of incubation, there were different levels of colorimetric absorbance observed. Figure 1 shows the relative fractional absorbance using the MTT assay after two additional days of incubation with various initial concentrations of Onc. Onc demonstrated a dose and time dependence of an inhibitory effect on relative fractional absorbance. The IC_{50} values (Onc concentration to inhibit the growth rate at 50%) were for FSaII 15 μg/ml, MCaIV >25 μg/ml, H4IIE 5 μg/ml, DU145 2 μg/ml, and CCL-209 1.5 μg/ml. Therefore, the CCL-209 endothelial cell line was the most sensitive to Onc among the exponentially growing cell lines tested.

Based on the screening of cytostatic/cytotoxic effects of Onc using the MTT assay, the clongenic assays of eight cell lines were performed after exposure to Onc 10 μg/ml for 24 h (Fig. 2). After 24 h incubation to Onc, all eight cell lines demonstrated variable sensitivity. Inter-

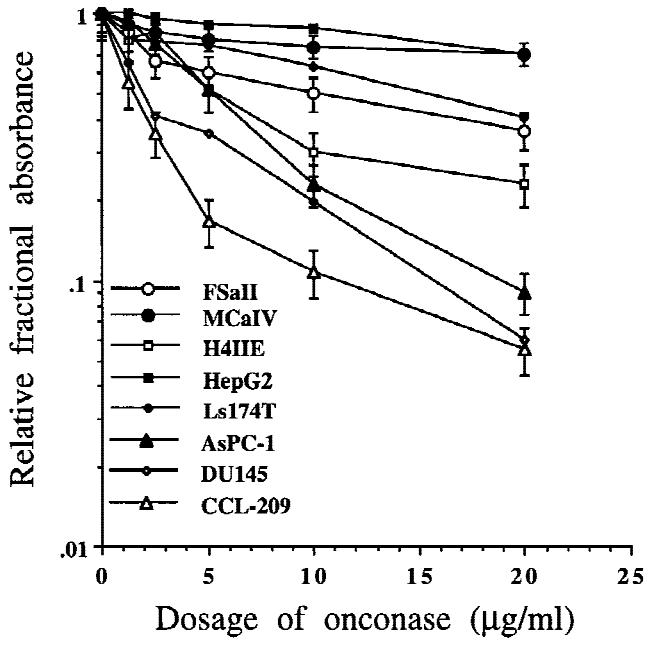

Fig. 1. The effect of Onc on relative fractional absorbance measured by the MTT assay. The proper cell number (5×10^4 to 1×10^5) was preincubated in 24-well cell culture plates overnight, then treated with fresh media containing 0–20 μg/ml of Onc for 24 h. The MTT assay was performed after an extended incubation (2 days) following removal of Onc (n = 4).

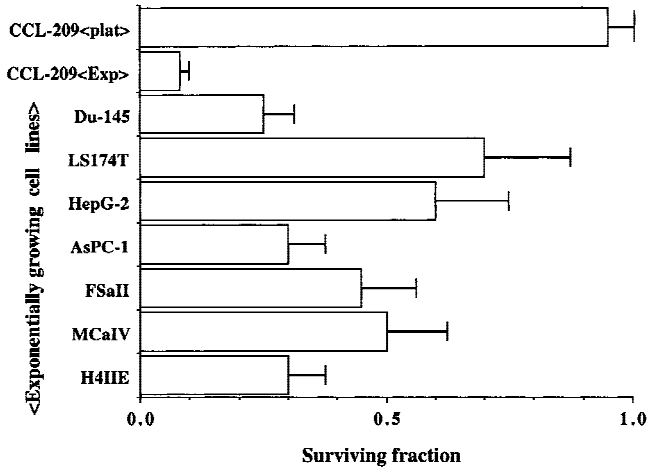

Fig. 2. The cell surviving fraction from various exponentially growing cell lines after exposure to Onc 10 μg/ml for 24 h. Cells (2×10^5 in T-25 cell culture flasks) were preincubated overnight, exposed to Onc 10 μg/ml for 24 h (n = 3), and trypsinized and replated in cell culture dishes with the proper cell number. In the case of CCL-209 cells, ⟨plat⟩ indicates confluent or plateau-phase growing cells and ⟨Exp⟩ indicates exponentially growing cells.

estingly, CCL-209 endothelial cells were the most sensitive to Onc among the eight cell lines studied. However, Onc was not effective in eliminating CCL-209 cells growing in the plateau-growth phase (i.e., no changes in viability, cell number, and plating efficiency). We did not see the differential inhibitory effect of Onc on the plateau-growth phase of tumor cell lines tested. Two human tumor cell lines, DU145 prostatic and AsPC-1 pancreatic carcinoma, and H4IIE rat hepatoma were sensitive to Onc. Two murine tumors, FSaII and MCaIV, were resistant to Onc. Ls174T human colon adenocarcinoma was the most resistant to Onc.

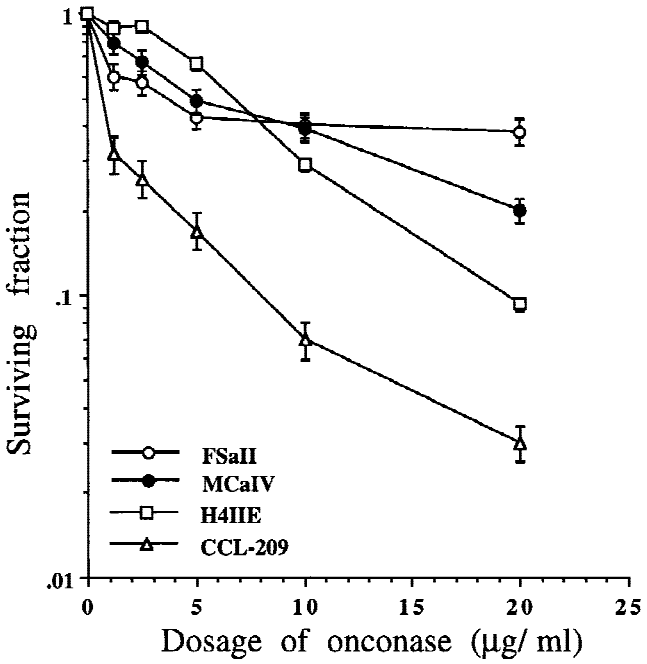

Fig. 3. The cell surviving fraction after exposure to Onc ranging from 0 to 20 μg/ml for 24 h. Cells (2×10^5 in T-25 cell culture flasks) were preincubated overnight, exposed to Onc (n = 3), and trypsinized and replated in cell culture dishes with the proper cell number in dishes.

The clonogenic survival of the four cell lines (FSaII, MCaIV, H4IIE, and CCL-209) were studied using various dosages of Onc (Fig. 3). The clonogenic assay was performed after exposure to Onc for 24 h. Both FSaII and MCaIV tumor cells were resistant to Onc at concentrations ranging from 5 to 20 μg/ml. However, the cytotoxicity of Onc against the H4IIE and CCL-209 cells was dose dependent. The survival curve for DU145 tumor cells was almost identical to that for CCL-209 (data not shown). Thus, the patterns of cytotoxicity of Onc were similar in the MTT and clonogenic assays.

In order to assess the effectiveness of Onc in inhibiting the growth of solid tumors in vivo, we performed the growth delay assay using four tumor models. Two (FSaII, MCaIV tumors) were Onc resistant in vitro (IC_{50} values >10 μg/ml) and the other two (H4IIE, DU145 tumors) were Onc sensitive in vitro (IC_{50} values <5 μg/ml). $LD_{10(30)}$ and $LD_{50(30)}$ of Onc for C3H mice were ~12.5 mg/kg and ~15 mg/kg, respectively. Based on our animal toxicity study of Onc, the maximum tolerated

TABLE I. Effect of Onc on the Growth Delay of Various Solid Tumors

Dose of Onc (mg/kg)[a]	Tumors	Tumor volume (mm^3)[b]	Growth delay (days)[b]
C3H mice			
2.5	FSaII	~200	0.5
5	FSaII	~240	1.5
10	FSaII	~250	5*
3 × 2.5[c]	FSaII	~200	3*
3 × 2.5[c]	MCaIV	~200	7*
Rats			
3 × 2.5[c]	H4IIE	~750	5*
Nude mice			
5	DU145	~230	12.5*

[a]Onc was administered ip.
[b]Growth delay was calculated by days required to reach 4 × the original tumor volume (n = 10–14 animals).
[c]Multiple injections for Onc were done three times (once per day) on days 0, 3, and 6.
*Statistically significant (vs. saline, $P < 0.05$).

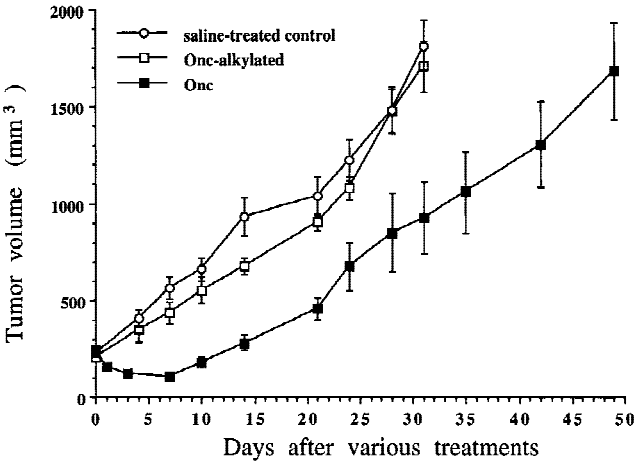

Fig. 4. The in vivo growth pattern of DU145 human prostatic tumor xenografts in nude mice after various treatments. The effect of Onc or alkylated Onc 5 mg/kg on inhibiting tumor growth (n = 10 mice per group). The alkylation of Onc was done with iodoacetate, which dramatically decreased its ribonucleic activity.

dosage of a single ip injection was 10 mg/kg. Both single and multiple ip injections of Onc were effective in inhibiting the growth of all four transplanted tumors (Table I). In FSaII tumors, the growth delay by a single ip injection of Onc 10 mg/kg (i.e., maximum tolerated dosage for C3H mice) was about 5 days; the growth delay by multiple ip injections (days 0, 3, and 6) of Onc 2.5 mg/kg was about 3 days. We observed that DU145 tumors were sensitive to Onc in vitro; therefore, instead of multiple injections of Onc, a single ip injection was utilized to test Onc's effectiveness in vivo. As expected, a single treatment of Onc 5 mg/kg significantly inhibited tumor growth of DU145 in nude mice (Fig. 4). However, alkylated Onc did not show anti-tumoral effects with the same dosage range of Onc.

To evaluate the possibility of reduction of TIFP by Onc, we measured TIFP (Table II). In FSaII, MCaIV, H4IIE, and DU145 tumors, median TIFPs were about 7, 8, 9, and 10 mm Hg, respectively. Multiple injections of Onc significantly decreased TIFP when the second TIFP measurements were performed 4 days after the first TIFP measurements. In FSaII tumors, Onc significantly ($P = 0.02$) decreased the median TIFP from about 7 mm Hg to about 3.5 mm Hg. We also observed that Onc significantly reduced TIFP for 7 days in MCaIV, H4IIE, and DU145 tumors.

MABP (baseline prior to Onc treatments) in anesthetized C3H mice was approximately 75 mm Hg. A single ip injection of Onc did not alter MABP and TIFP in C3H mice bearing either FSaII (data not shown) or MCaIV (Fig. 5) tumors within 2 h. Although we did not observe any acute effects of Onc on TIFP in MCaIV tumors, TBF increased significantly between 30 and 60 min after treatment with ONC (Fig. 5). However, when the chronic

TABLE II. Effect of Onc on TIFP of Various Solid Tumors

Dose of Onc (mg/kg)[a]	Tumors (n = 7 per group)	TIFP Day 0	Day 1	Day 4	Day 7	Day 10
C3H mice						
5	FSaII	7	4*	6	7.5	ND
5	MCaIV	8	5*	6.5	7	ND
3 × 2.5[b]	FSaII	7	ND	3.5*	2*	6
3 × 2.5[b]	MCaIV	8	ND	6	3*	5.5*
Rats						
3 × 2.5[b]	H4IIE	9	ND	5*	3*	6.5
Nude mice						
5	DU145	10	5*	5*	4*	8

[a]Onc was administered ip.
[b]Multiple injections for Onc were done three times (once per day) on days 0, 3, and 6.
*Statistically significant (vs. day 0, $P < 0.05$); ND = not determined.

effect of Onc on TIFP was monitored, the TIFP in FSaII, MCaIV, and DU145 tumors was reduced by a single injection of Onc 5 mg/kg by about 24 h posttreatment (Table II).

In view of our previous studies [13], untreated control FSaII tumors were very hypoxic, as reflected by low tumor oxygen levels. Thus, we chose MCaIV tumors for further measurements of TBF and pO_2 after treatment with Onc. At a dose of 5 mg/kg (or about 100 μg per mouse), Onc significantly improved tumor oxygenation in MCaIV tumors, when intratumoral pO_2 was measured between 30–60 min postinjection of Onc (Fig. 6). Median pO_2 increased from 10 to 15 mm Hg ($P = 0.001$), and percent of readings of intratumoral pO_2 values below 2.5 mm Hg decreased from 13.5% to 4.3%. A slight decrease in pO_2 was observed 6 h posttreatment com-

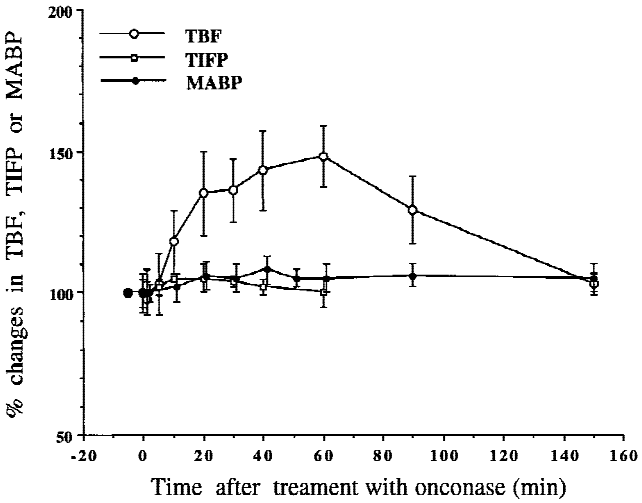

Fig. 5. Percent changes in TBF, TIFP, and MABP after a single ip injection of Onc 5 mg/kg in C3H mice bearing MCaIV tumors (n = 7–10 mice). Immediate reductions in MABP and TIFP after treatment with Onc were not observed. However, TBF significantly increased during 2 h of observation, returning to the original level within about 3 h. The solid circles represent the fluctuation of MABP after an ip administration of saline (0.9% sodium chloride solution) 10 ml/kg.

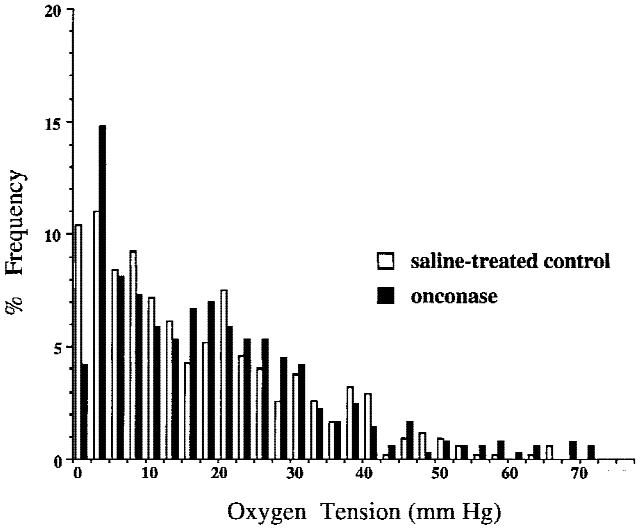

Fig. 6. Frequency distribution of pO_2 in MCaIV tumors (n = 10 mice per group; mean tumor volume = about 250 mm^3) after a single ip injection of saline (0.9% sodium chloride solution) or Onc 5 mg/kg. Median pO_2 value for the saline-treated control from 370 individual pO_2 readings was 10 mm Hg, of which 13.5% of the readings were <2.5 mm Hg. Onc significantly increased median intratumoral pO_2 to 15 mm Hg (from 365 pO_2 readings). Percent of the pO_2 readings with <2.5 mm Hg was 4.5%.

pared to 1 h posttreatment. However, Onc continuously improved tumor oxygenation when intratumoral pO_2 was measured during a 48 h period (i.e., median pO_2 at 48 h = 17 mm Hg; Fig. 7).

DISCUSSION

The first goal of this study was to determine if Onc would inhibit cell growth and clonogenicity of several

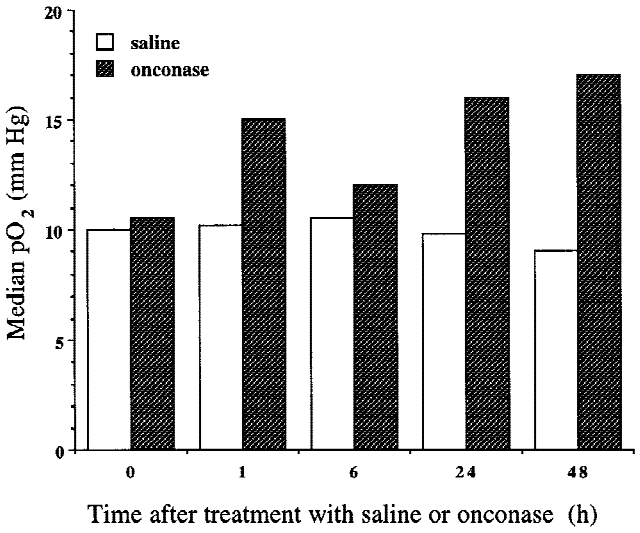

Fig. 7. Changes in median intratumoral pO_2 after an ip injection of either saline (0.9% sodium chloride solution) 10 ml/kg or Onc 5 mg/kg (n = 10 mice per time point).

cell lines in vitro. When MTT assays were performed 2 days after the removal of Onc, the degree of cytotoxicity of Onc estimated by the MTT and clonogenic assays was similar (Fig. 1). When the cell viability was determined using a trypan blue exclusion assay, we observed significant reduction in both cell numbers and the viability during this extended incubation of 2–5 days. Although we did not observe DNA fragmentation using gel electrophoresis (Lee et al., unpublished data, 1998), some portions of the cells were unable to exclude trypan blue, indicating that membrane integrity was damaged. Further quantitative evaluation using in situ fluorescent techniques is warranted to clarify the cellular mechanism of the cytotoxic effect of Onc on the induction of apoptosis. Lee and Biaglow (unpublished data, 1999) observed that the percent of apoptosis induced by Onc increased from about 2% to about 15% in K1 CHO parent cell lines using nuclear fragmentation as the apoptotic response, stained with propidium idodide, and assessed by fluorescent microscopy.

This study has demonstrated the cytotoxicity of Onc on eight exponentially growing cell lines, estimated by both the MTT and clonogenic assays. Among the eight cell lines studied, the CCL-209 cells were the most sensitive to Onc. It is well known that many chemotherapeutic agents target cycling cells. When the endothelial cells were exposed to Onc for more than 48 h during the exponential-growth phase, Onc completely inhibited their growth. In contrast, the confluent (or plateau-growth phase) endothelial cells were not effectively killed by Onc (Fig. 2). Our in vivo studies of Onc in various tumor implanted sites indicate (Lee et al., unpublished data, 1998) that an intratumoral injection of Onc into FSaII tail tumors induced complete vascular destruc-

tion of tumors. This indicates that Onc may have more cytotoxicity and specificity against the exponentially growing endothelial cells or it may demonstrate an otherwise mechanistically undefined vascular-targeting activity. The mechanism of action notwithstanding, Onc may be a good candidate to be used as a new vascular-targeting agent.

The second goal of this study was to determine if Onc inhibits the growth of solid tumors in vivo. Onc was effective in inhibiting the growth of all four tumor models studied (Table I). In vitro cell growth inhibition by Onc might be extrapolated to some degree into the inhibition of tumor growth in solid tumors in vivo. To our knowledge, it is the first study of anti-tumoral effects of Onc on various solid tumors. Onc was less cytotoxic to both FSaII and MCaIV tumor cells than DU145 and H4IIE tumor cells in vitro (Figs. 1–3), and was most effective against the DU145 tumors in vivo, as it might be anticipated from our in vitro studies.

When 9L glioma was treated with the combination of 2-deoxy glucose (known as a glycolysis inhibitor) and NaN_3 (known as an oxidative phosphorylation inhibitor), Onc-induced inhibition of protein synthesis was completely blocked. Thus, Wu et al. [1] concluded that endocytosis, which requires adenosine triphospahte (ATP), may be a step involved in Onc cytotoxicity. The ribonucleolytic activity of Onc also seemed to be essential for its cytotoxicity. The alkylation of Onc with iodoacetate dramatically decreased its ribonucleic activity. As a result, alkylated Onc, which only retained 2% of its ribonucleolytic activity, was about 100-fold less potent in inhibiting protein synthesis in 9L glioma in vitro [1]. We also found that alkylation of Onc caused loss of the tumoricidal effects of Onc in vivo (Fig. 4). Therefore, this ribonucleolytic activity appears necessary for the tumoricidal effect of Onc in vivo.

The third goal of our study was to test if Onc could lower the TIFP in solid tumors. Elevated TIFP is known to be a pathophysiological characteristic of rodent and human solid tumors [13,14]. It is also believed to be a major obstacle to the delivery of macromolecules into solid tumors. Onc significantly decreased TIFP ($P = 0.02$), reaching about 50% of the control value in FSaII tumors (Table II). We previously demonstrated that nicotinamide and pentoxifylline decrease TIFP in mice bearing FSaII tumors [5,13]. In this study, Onc has been shown to alter TIFP in various tumor models in mice and rats. As shown in Figure 5, Onc did not affect the MABP. Thus, it is possible that the decrease in TIFP was caused by an increase in the hydraulic conductivity of the interstitial space. If microvascular pressure (MVP) was not modified by Onc, a pressure gradient would be created by the reduction of TIFP. The pressure gradient could favor the extravasation of macromolecules by convection. However, it is also possible that the decrease in TIFP was due to the reduction in MVP. As previously proposed by several investigators [5–9,12,13], with regard to the treatments with several physical or chemical therapeutic compounds in murine or human tumors, the changes in TIFP by Onc may serve as a quantitative indicator to predict penetrability by Onc and/or other molecules in solid tumor tissues in vivo.

The mechanism of the acute effect of Onc on tumor vasculature is not known. However, as shown in Figure 6, median pO_2 increased from 10 to 15 mm Hg, and percent readings of pO_2 below 2.5 mm Hg decreased from 13.5% to 4.3%. The improved tumor oxygenation was associated with a statistically significant increase in RBC flux in the center of tumors (Fig. 5). This acute effect may be due to reduction in viscous resistance. On the other hand, the chronic effect of Onc on tumor vasculature may be due to reduction in combined manipulation of both viscous and geometric resistance, where both types of resistance were comparatively elevated in tumors. Geometric resistance to blood flow increased with the number of excess blood vessels [15]. An increased resistance contributed to the elevated TIFP in solid tumors. Due to the inhibition of the cell proliferation and clonogenic cell survival of endothelial cells by Onc, the O_2 consumption significantly diminished, making O_2 more available to the perivascular tissues. Onc was also directly cytotoxic to cancer cells. Fewer viable cells resulted in a decreased O_2 consumption without changes in O_2 availability through a better blood supply. We also postulate that chronic effects of Onc may inhibit the vascular redundancy that is a hallmark of angiogenesis. This contributes to the enlargement of the remaining vessels, resulting in a decrease in geometric resistance and an improved blood flow. If this is the case, Onc would improve drug and oxygen delivery as a result of augmented blood flow and oxygenation, accompanied by decreased TIFP (Fig. 7). We recently observed that the oxygen consumption rate (QO_2) in 9L tumors and K1 CHO parent cell lines was significantly decreased by Onc 10 μg/ ml (Lee et al., unpublished data, 1999).

CONCLUSIONS

This study has demonstrated that the pathophysiological effects of Onc significantly contributed to its overall biological activity in vivo. It has shown anti-tumoral effects against various tumors in vitro as well as in vivo. We have also provided some insight regarding its potential physiological benefit as a new therapeutic agent in cancer treatment. Furthermore, the study of the radiation response in vivo induced by Onc warrants testing if Onc can be a novel radiation enhancer, due to its effects on increases in both TBF and intratumoral pO_2 based on our present studies.

ACKNOWLEDGMENTS

This study was supported by Alfacell Corporation, Bloomfield, NJ [to IL] and UMDNJ-Robert Wood Johnson Medical School, Camden, NJ [to IL]. Dr. I. Lee sincerely appreciates the financial support provided by Dr. W. Gilles McKenna, Chairman, Department of Radiation Oncology, University of Pennsylvania School of Medicine.

REFERENCES

1. Wu YN, Mikulski SM, Ardelt W, et al.: A cytotoxic ribonuclease study of the mechanism of onconase cytotoxicity. J Biol Chem 1993;268:10686–10693.
2. Lin JJ, Newton DL, Mikulski SM, et al.: Characterization of the mechanism of cellular and cell free protein synthesis inhibition by an anti-tumor ribonuclease. Biochem Biophys Res Commun 1994; 204:156–162.
3. Mikulski SM, Berstein EH, Ardelt W, et al.: Striking increase of survival of mice bearing M109 Madison carcinoma treated with a novel protein from amphibian embryos. J Natl Cancer Inst 1990; 82:151–153.
4. Jain RK: Barriers to drug delivery in solid tumors. Sci Am 1994; 271:58–65.
5. Lee I, Boucher Y, Jain RK: Nicotinamide can lower tumor interstitial hypertension: Mechanistic and therapeutic implications. Cancer Res 1992;52:3237–3240.
6. Curti BD, Urba WJ, Alvord WG, et al.: Interstitial pressure of subcutaneous nodules in melanoma and lymphoma patients: Changes during treatment. Cancer Res 1993;53:2204–2207.
7. Leunig M, Goetz AE, Dellian M, et al.: Interstitial fluid pressure in solid tumors following hyperthermia: Possible correlation with therapeutic response. Cancer Res 1992;53:487–490.
8. Leunig M, Goetz AE, Gammara F, et al.: Tumor interstitial fluid pressure and volume changes following photodynamic therapy. Br J Cancer 1994;69:101–103.
9. Roh HD, Boucher Y, Kalnicki S, et al.: Interstitial hypertension in carcinoma of uterine cervix in patients: Possible correlation with oxygen tension and radiation response. Cancer Res 1991;51: 6695–6698.
10. Mitchell JB, Bedford JS, Bailey SM: Dose-rate effects on the cell cycle and survival of S3 Hela and V79 cells. Radiat Res 1979; 79:520–536.
11. Pagliacci MC, Spinozzi F, Migliorati G: Genistein inhibits tumor cell growth in vitro but enhances mitochondria reduction of tetrazolium salts: A further pitfall in the use of the MTT assay for evaluating cell growth and survival. Eur J Cancer 1993;294:1573–1577.
12. Lee I, Demhartner TJ, Boucher Y, et al.: Effects of hemodilution and resuscitation on tumor interstitial fluid pressure, blood flow, and oxygenation. Microvasc Res 1994;48:1–12.
13. Lee I, Boucher Y, Demhartner TJ, et al.: Changes in tumor blood flow, oxygenation and tumor interstitial fluid pressure induced by pentoxifylline. Br J Cancer 1994;69:492–496.
14. Boucher Y, Lee I, Jain RK: Lack of general correlation between interstitial fluid pressure and oxygen partial pressure in solid tumors. Microvasc Res 1995;50:175–182.
15. Jain RK: Determinants of tumor blood flow: A review. Cancer Res 1988;48:2641–2658.